

Scientific journey to the first FDA-approved drug for eosinophilic esophagitis



Marc E. Rothenberg, MD, PhD Cincinnati, Ohio

When eosinophilia was first associated with esophagitis, it was thought to reflect gastroesophageal reflux disease, especially given the efficacy of reflux medications to abate esophageal eosinophilia in many individuals. Subsequent studies demonstrated disease remittance with amino acid–based formulas and conversely induction of esophageal eosinophilia in mice following allergen challenge. These results, along with the finding that proton pump inhibitors alleviated esophageal eosinophilia by an anti-inflammatory mechanism, turned attention away from an acid-induced pathogenesis and established eosinophilic esophagitis (EoE) as a separate disease entity driven by allergic inflammation. The disease underpinnings were elucidated by analysis of esophageal transcriptomic profiling, revealing gene signatures orchestrated by type 2 cytokine signaling, mainly IL-13. Preclinical studies showed that IL-13 overproduction was sufficient to induce EoE-like changes in mice and human *ex vivo* systems and conversely that inhibiting IL-13 signaling attenuated these processes. An early proof-of-principle study with a humanized anti-IL-13 mAb in patients with EoE revealed correction of the EoE transcriptome and attenuation of esophageal eosinophilia, providing a rationale for advancing anti-type 2 cytokine therapy for EoE. Dupilumab, a precision therapeutic mAb that blocks the shared IL-13 and IL-4 receptor, is the first drug to advance through clinical trials and receive US Food and Drug Administration approval for EoE. The ability of dupilumab to

improve clinical, histologic, endoscopic, and molecular features of EoE and garner US Food and Drug Administration approval is a victory for science, rare diseases, patients, and advocacy and provides a framework for developing additional EoE treatments and approved treatments for eosinophilic gastrointestinal disease beyond the esophagus. (J Allergy Clin Immunol 2022;150:1325-32.)

Key words: Discovery, dupilumab, eosinophils, esophagitis, FDA, IL-4, IL-5, IL-13

CHALLENGING THE CONVENTIONAL WISDOM THAT EoE IS NOT ACID REFLUX DISEASE

The main tissue reservoir for eosinophils is the gastrointestinal tract, yet the esophagus is the only gastrointestinal segment devoid of eosinophils.¹ As such, detection of esophageal eosinophilia nearly always denotes pathology.² When I was in training, the early views were that esophageal eosinophilia was diagnostic for gastroesophageal reflux disease.³ However, the finding that the proximal esophagus was prominently involved and the subsequent ability of amino acid–based formulas to reduce esophageal eosinophilia prompted consideration of a nonallergic disease etiology, perhaps involving glucocorticoid-responsive immunologic hypersensitivity to food.⁴⁻⁷

A definitive role for allergic inflammation in the etiology of eosinophilic esophagitis (EoE) was supported by our finding that allergen exposure in mice was sufficient to experimentally induce an EoE-like response.⁸ Disease pathology was eotaxin and IL-5 dependent, and an intimate connection between respiratory and esophageal inflammation was demonstrated. In parallel, clinical studies suggested the involvement of T cells and IL-5 in human esophageal biopsy specimens from patients with EoE.⁹ These collective findings shifted attention away from an acid-induced mechanism to the role of type 2 immunity.

PRECLINICAL STUDIES DREW ATTENTION TO EoE BEING MEDIATED BY TYPE 2 IMMUNITY

Our early work demonstrated that intratracheal delivery of IL-13 was sufficient to induce esophageal eosinophilia in mice.¹⁰ The effect of IL-13 was dose and concentration dependent; it involved a mechanism dependent on STAT6, IL-5, and eotaxin-1 and was relatively specific, as other cytokines tested (IL-4, IL-9, and IL-10) did not elicit this response.¹⁰ Conversely, we showed that allergen-induced experimental EoE was attenuated in mice engineered to be deficient in these type 2 cytokines and/or *stat6*.¹¹ Putting these murine findings together, we then demonstrated

From the Division of Allergy and Immunology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine.

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Corresponding author: Marc E. Rothenberg, MD, PhD, Division of Allergy and Immunology, Cincinnati Children's Hospital Medical Center, Department of Pediatrics, University of Cincinnati College of Medicine: 3333 Burnet Ave, ML 7028, Cincinnati, OH 45229. E-mail: Rothenberg@cchmc.org.

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Abbreviations used

EoE: Eosinophilic esophagitis
 FDA: US Food and Drug Administration
 PAG: Patient advocacy group

that a human anti-human IL-13 mAb (CAT-354) could reverse EoE-like changes induced by human IL-13 in mice.¹² CAT-354 is now approved for atopic dermatitis under the name tralokinumab.¹³ Furthermore, we discovered that mice genetically engineered to overexpress IL-13 using a lung promoter developed profound EoE-like changes.¹⁴ IL-13 kinetically accumulated in the esophagus of these mice (following induction of transgene expression), and over the course of only several weeks, it was associated with esophageal eosinophilia, epithelial hyperplasia, collagen deposition, and angiogenesis, features that occur in human EoE (Fig 1). Interestingly, the effect of IL-13 was so profound that there were visible changes of esophageal edema and lengthening of the esophageal circumference, reminiscent of the gross endoscopic findings seen in patients with EoE. Mechanistically, esophageal eosinophilia was eotaxin-1-dependent, but interestingly, both eosinophils and eotaxin-1 were dispensable for the pathologic features of the disease, consistent with

subsequent studies, which have so far failed to demonstrate clinical benefit of anti-eosinophil therapy in human EoE.^{15,16} Furthermore, the activity of proton pump inhibitors in EoE was recognized to work by a mechanism beyond inhibiting acid production and instead by a mechanism mediated by its anti-inflammatory effect, including by inhibiting eotaxin production, and it was most recently demonstrated to be mediated by the aryl hydrocarbon receptor.¹⁷⁻¹⁹ Collectively, these studies drew significant attention to the role of type 2 immunity, particularly IL-13, in the potential pathobiology of EoE. Furthermore, these studies suggested that eosinophils may be a byproduct of the type 2 immune response rather than a key orchestrator of esophageal pathobiology. These studies focused attention on the epithelial expression and signaling of the IL-13 receptor, composed of the type 2 IL-4 receptor (IL-4 receptor α -chain [IL-4R α] and IL-13R α 1), and IL-13R α 2, the latter being inhibitory in the esophagus (Fig 2).¹⁴

HUMAN TRANSLATIONAL STUDIES SUBSTANTIATED THAT IL-13 MAY MEDIATE EoE

EoE provided an opportunity to probe human allergic inflammation at a level not previously achieved, as biopsy specimens were readily available for research investigation. Prior studies had primarily researched biologic specimens from a remote tissue (the

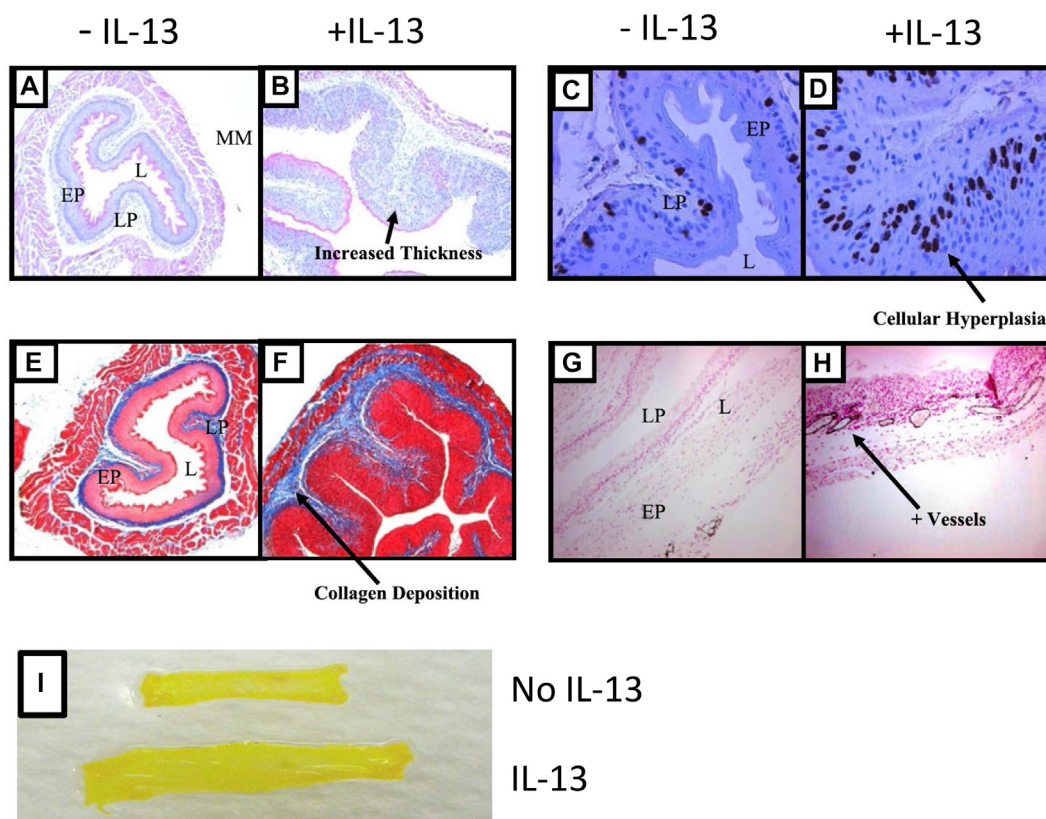


FIG 1. IL-13 transgene-induced esophageal tissue remodeling. The esophagus of IL-13 transgenic mice in the absence (A, C, E, and G) and presence of IL-13 induction (B, D, F, and H) for 4 weeks. Esophageal sections were stained with hematoxylin and eosin (A and B), for a marker of epithelial proliferation by BrdU incorporation (C and D), for collagen deposition by trichrome staining (E and F), and for a marker of angiogenesis with anti-PECAM-1 (G and H). Enlarged esophageal circumference (I) visualized in an excised cross section of the esophagus in the presence and absence of IL-13 exposure *in vivo*. EP, Epithelial; L, lumen; LP, lamina propria; MM, muscularis mucosa. Adapted with permission.¹⁴

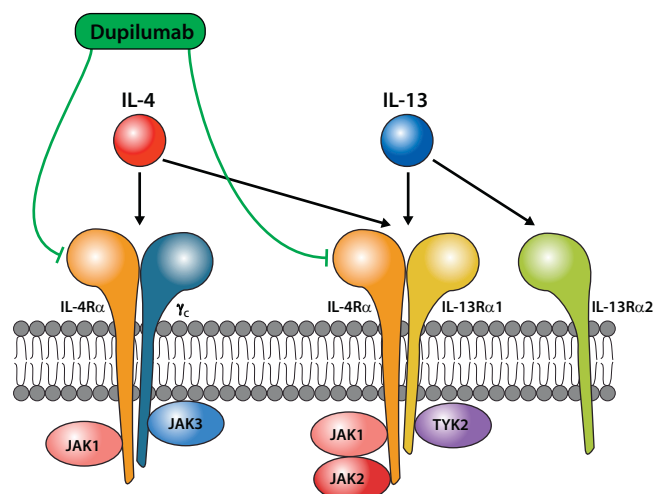


FIG 2. Schematic diagram of IL-4 and IL-13 receptors and the impact of dupilumab. IL-4 and IL-13 receptors and the effect of dupilumab as an antibody that blocks IL-4R α are shown. The IL-4 receptor is composed of 2 transmembrane proteins. IL-4R α binds IL-4 with high affinity, leading to dimerization with either the common γ -chain (γ C) or IL-13R α 1, forming the type I or type II receptor complex, respectively. IL-13 binds to IL-13R α 1 with lower affinity, followed by heterodimerization with IL-4R α to form a high-affinity complex. IL-13 also binds to IL-13R α 2, which is thought to serve as a “decoy receptor” in the esophagus. The interaction of the receptor components with the Janus kinase (JAK) signaling molecules is shown.

blood), difficult-to-obtain bronchoalveolar lavage fluid, or biopsy specimens from the lung or skin. The ability to safely and routinely obtain millimeter-sized endoscopic biopsy specimens from the esophagus, even from children, allowed us to apply early genome-wide expression profiling technologies to study human allergic inflammation.²⁰ Computational analysis of more than 50,000 esophageal transcripts identified dysregulated expression of approximately 1% of the human genome in active biopsy specimens. I was convinced of the value of this approach when our initial analysis discovered that eotaxin-3, a potent chemoattractant and activating factor for eosinophils, was the most upregulated gene in the whole human genome in the esophagus of patients with active disease.²⁰ We were struck by the finding that the EoE transcriptome was enriched for known IL-13-regulated genes, including eotaxin-3. Using primary esophageal epithelial cells derived from EoE biopsy specimens (or an immortalized esophageal epithelial cell line), we were able to show that this single cytokine (IL-13) was sufficient to induce a substantial segment of the EoE transcriptome *ex vivo*, including eotaxin-3 as the most strongly upregulated gene.²¹ IL-13 was sufficient not only to induce many of the EoE transcripts but also to downregulate esophageal epithelial barrier function, including loss of filaggrin and desmosome genes,²¹⁻²³ processes that are germane to the pathoetiology and genetic susceptibility of EoE.²⁴ The ability to recapitulate the human EoE transcriptome with a single cytokine (IL-13) and a single cell type (esophageal epithelial cells) provided the framework for the eventual view that EoE pathogenesis involved the central interplay of adaptive IL-13⁺ memory T cells with innate IL-13R α 1⁺ epithelial immunity.^{25,26} Indeed, genome-wide association studies have revealed that the chief EoE susceptibility loci are located at 2p23 and 5q22, regions that encode for the key epithelial gene products calpain-14 and thymic stromal lymphopoietin, respectively.²⁷⁻³⁰ Calpain-14 is esophageal

specific; it regulates barrier function, and importantly, it is transcriptionally activated by IL-13 at extraordinary levels comparable to the activation levels of eotaxin-3.^{28,31} Thymic stromal lymphopoietin is a key pro-atopy alarmin released from breached epithelial cells and is overproduced in the esophagus of patients with EoE.^{27,32,33} Recent single-cell RNA sequencing analysis of esophageal biopsy specimens, particularly focused on T cells and mast cells, localized IL-13 production to pathogenic effector memory IL-5⁺IL-13⁺ CD4⁺ T cells, which also express the free fatty acid receptor (FFAR3) and GPR15, as well as esophageal resident mast cells.^{25,34,35} Furthermore, we have shown that IL-13 is able to directly mediate other features of EoE *in vitro*, including esophageal epithelial cell proliferation, loss of esophageal epithelial differentiation, and profibrotic processes in esophageal endothelial cells, including loss of tetraspan protein 12 (TSPAN12).^{21,33,36} These collective human translational findings, buttressed with the preclinical murine findings, provided substantial evidence for the key role of IL-13 in the development of EoE.

EARLY PROOF-OF-PRINCIPLE CLINICAL TRIAL WITH ANTI-IL-13 IN HUMANS

To prove the theory that EoE was mediated, at least in part, by IL-13, I convinced a pharmaceutical company to allow us to study their humanized anti-human IL-13 mAb (QAX576) in patients with EoE. In a single-site study, we treated 23 adult patients with EoE with 3 intravenous doses of QAX576 or placebo over the course of 3 months.³⁷ Patients treated with the drug had statistically reduced esophageal eosinophilia and a trend for improved symptoms, although a non-disease-specific patient-reported outcome metric was used to measure symptoms (the Mayo Dysphagia Questionnaire). At the same time, we examined the EoE transcriptome before and after treatment and were impressed by the profound reversibility of the molecular markers of the disease, even 6 months after cessation of drug therapy. This allowed us to construct a functional map of IL-13-dependent nodes in humans within the context of allergic esophageal inflammation. This map was notably enriched in chemokines, proteases, barrier function, mast cell signature genes, and tissue remodeling pathways, which we attributed to IL-13-induced responses (Fig 3). This relatively small-scale, single-site study, which was carefully planned to examine key molecular biomarkers, provided the proof-of-principle that would propel multiple pharmaceutical companies to pursue larger-scale and eventual registration trials with their relevant drugs.

KEY PARTNERSHIPS AND ADVOCACY

Motivated by the need to find answers for their children with eosinophil-associated gastrointestinal diseases, several patient advocacy groups (PAGs) were formed in the United States; they included the American Partnership for Eosinophilic Diseases, Campaign Urging Research for Eosinophilic Diseases, and Eosinophil Family Coalition. These groups have been essential in raising awareness about EoE and related eosinophilic diseases, providing education about these diseases (through their websites, meetings, and media), raising substantial research funds, and promoting legislation (such as medical formula insurance coverage and enactment of National Eosinophil Awareness Week by the US Congress). Medical researchers, PAGs, and other key stakeholders in the EoE field were successful in

Progress Toward FDA Approval

Field Advances Across the Years

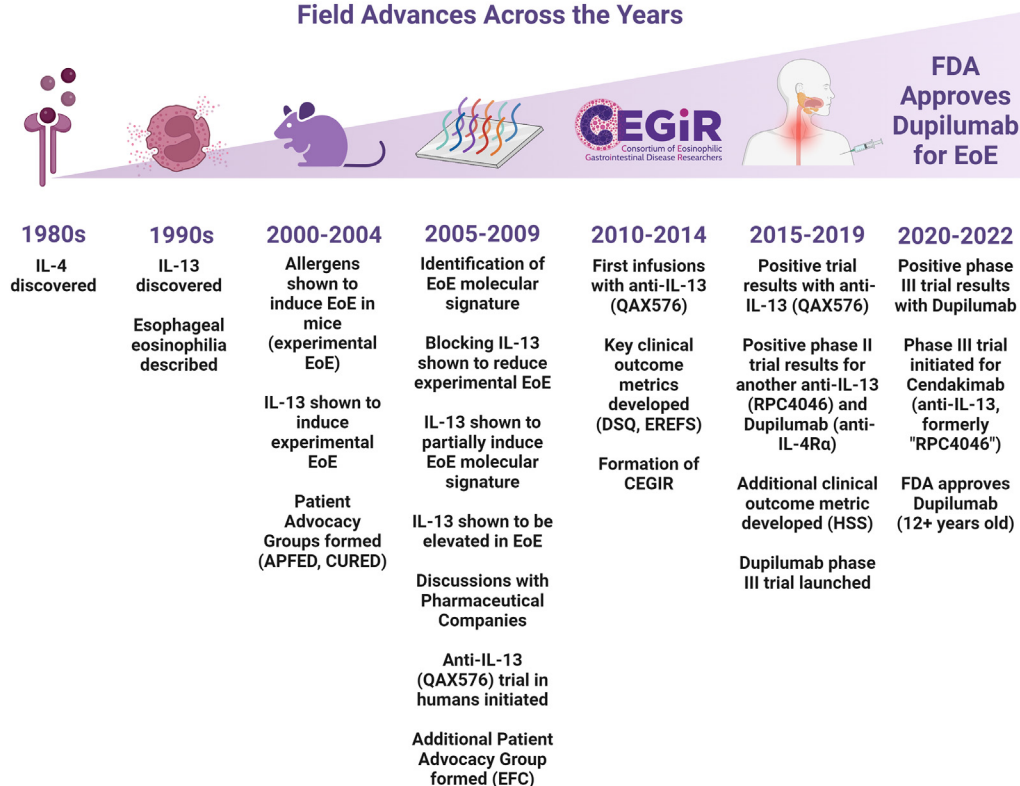


FIG 4. Time line of relevant events for FDA approval of dupilumab for EoE. From the discovery of IL-4 in the 1980s to anti-interleukin clinical trials from the late 2000s onward, FDA approval of a drug for EoE has been decades in the making and the result of basic scientific advances and key partnerships and collaborations. Cendakimab is an anti-IL-13 antibody, formerly called RPC4046; dupilumab is an anti-IL-4 receptor α -chain (anti-IL-4R α) mAb, and IL-4R α is part of the cell receptor for the cytokine IL-13. *APFED*, American Partnership for Eosinophilic Diseases; *CEGiR*, Consortium of Eosinophilic Gastrointestinal Disease Researchers; *CURED*, Campaign Urging Research for Eosinophilic Diseases; *DSQ*, Dysphagia Symptom Questionnaire; *EFC*, Eosinophilic Family Coalition; *EREFS*, Endoscopic Reference Score; *HSS*, Histology Scoring System. Figure generated by Shawna Hottinger using Biorender.

EoE. Although mepolizumab and reslizumab lowered esophageal eosinophil levels, this effect was modest, and although clinical symptoms improved, a similar clinical response was seen in the placebo-treated patients.^{15,16,53} Notably, these studies were limited by not including many of the disease-specific clinical outcome metrics discussed earlier in this article, as they were not yet developed and/or recognized instruments. These trials, however, set the stage for researching the potential utility of other classes of biologic agents for the treatment of EoE.

On the basis of the emerging preclinical, translational, and genetic data and the readily available clinical outcome metric tools already discussed, pharmaceutical companies became enthusiastic to partner with my colleagues and me to conduct multisite, placebo-controlled trials for patients with EoE. Herein, I will focus only on dupilumab, although the clinical progress with another anti-IL-13 antibody, cendakimab, is positive and promising.⁵⁴⁻⁵⁷ Dupilumab is a VelocImmune-derived human mAb against IL-4R α , which inhibits IL-4 and IL-13 signaling. VelocImmune is technology for producing optimized fully humanized mAbs that utilizes a proprietary mouse platform engineered with a genetically humanized immune system. Dupilumab is approved for atopic dermatitis, eosinophilic

asthma, and chronic sinusitis with nasal polyposis. In an early phase 2 study, my colleagues and I were able to show positive results, with improvement in clinical symptoms and lowering of esophageal eosinophil levels in patients with EoE treated with weekly subcutaneous dupilumab compared with placebo.⁵⁸ These encouraging data led to a 3-part phase 3 registration trial called the LIBERTY EoE TREET study (ClinicalTrials.gov identifier NCT03633617), in which the efficacy and safety of dupilumab in adolescent and adult patients with EoE were studied in a randomized, double-blind, parallel-group, multicenter, placebo-controlled trial. The study included two 24-week treatment periods that were conducted independently in separate groups of patients (parts A and B), as well as a third part (part C), during which patients received weekly or biweekly dupilumab for an additional 24 weeks. In both parts A and B of the trial, about 60% of the patients who received dupilumab achieved a predetermined level of reduced eosinophils in the esophagus compared with about 5% of the patients who received placebo. Patients in both parts A and B who received dupilumab also experienced clinically significant improvement in symptoms (as measured by the Dysphagia Symptom Questionnaire instrument). Similarly, patients in part C demonstrated sustained improvements during

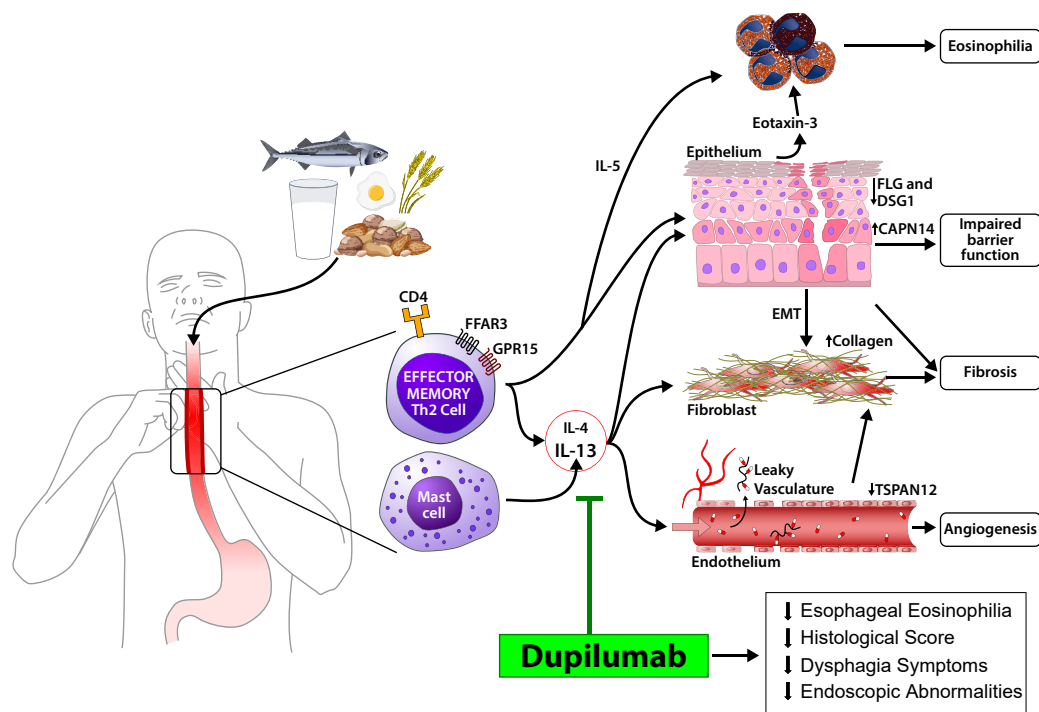


FIG 5. Schematic diagram of type 2 immunity in EoE and the impact of dupilumab. Food induces effector memory $CD4^+$ T_H2 cells that express the free fatty acid-binding receptor 2 (FFAR2) and G protein-coupled receptor 15 (GPR15). These esophageal resident cells, along with esophageal mast cells, produce large amounts of IL-5 and IL-13 and lesser amounts of IL-4. IL-5 stimulates eosinophilopoiesis and survival. IL-4 and IL-13 stimulate epithelial cells to produce eotaxin-3, which recruits and activates eosinophils. IL-4 and IL-13 also decrease filaggrin (FLG) and desmoglein 1 (DSG1) expression, thereby impairing barrier function. IL-4 and IL-13 stimulate fibroblast production of collagen and loss of TSPAN12 expression by endothelium, thereby contributing to tissue fibrosis, angiogenesis, epithelial cell mesenchymal transformation (EMT), and extracellular matrix (ECM) deposition.

the additional 24 weeks. All primary and secondary end points, except for symptom improvement requiring weekly dosing, were improved with weekly and biweekly dosing.^{59,60} This strong data set, demonstrating that dupilumab reduced esophageal eosinophilia, improved clinical symptoms, decreased endoscopic findings, and reversed key biomarkers (including elevated serum eotaxin-3 levels and the EoE transcriptome), led to victorious FDA approval of dupilumab as the first drug for EoE. Touchingly, the FDA approval occurred at the end of the National Eosinophil Awareness Week on May 20, 2022.

CONCLUDING REMARKS AND FUTURE STUDIES

The long and exciting scientific journey that led to the first FDA approval of a drug for EoE is summarized in Fig 4. This truly impactful advance started with immunology research nearly 40 years ago, which was well before EoE was even known to be a disease entity.^{61,62} Too often research is deemed valuable, especially by funding agencies, solely on the basis of its translational potential, but one of the lessons learned from the described journey is that basic research is important in its own right. My pursuit in understanding the pathogenesis of EoE and using this information to develop effective therapies was not a straightforward path; rather, it involved overturning conventional wisdom, drawing attention to a rare disease that was not previously appreciated, pursuing preclinical sciences (eg, murine modeling), learning and applying new technologies (eg, genomics and computational informatics),

taking risky bold steps before many others were interested in partnerships, engaging in dialog with the FDA, and importantly, developing key partnerships with industry, diverse scientists, and patients, especially through PAGs. This was not a sole venture; instead, it was completely dependent on working together with outstanding and committed investigators at many institutions, especially clinical scientists skilled at developing necessary outcome-assessing tools.

Transforming esophageal eosinophilia from a disease process thought to be a simple secondary response to acid reflux into a *bona fide* separate food allergy disease mediated by type 2 immunity (Fig 5) and having its own diagnostic code and criteria involved steps that were sometimes quick but too often slow (Fig 4). The journey assembled substantial coordinated efforts between diverse stakeholders, including scientists, clinicians, patients, PAGs, funding organizations (eg, the National Institutes of Health), industry partners, and regulatory authorities (eg, the FDA). Although the approval of dupilumab for EoE is a significant victory, there are still major outstanding unanswered questions concerning its use, including proper dosage, durability, long-term safety, predictions of responsiveness and resistance, and its impact on dietary restrictions and natural history. Furthermore, determining its efficacy and safety in young children with EoE is an active area of focus (ClinicalTrials.gov identifier NCT04394351), especially in view of the frequent early-onset form of the disease.⁶³ The FDA approval of EoE is a success for collaborative science, the National Institutes of Health,

patients, rare diseases, and the involved pharmaceutical companies. The journey encountered and the obstacles overcome provide a now quickened pathway forward for new drug approvals for EoE and other rare diseases.

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